ORIGINAL ARTICLE

In Vitro Mutagenicity Evaluation of PM_1 and $PM_{0.1}$ Air Particulates in the Urban Area of Malaysia

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ABSTRACT

Introduction: Particulate matter (PM) with smaller aerodynamic diameter such as the PM_1 and $PM_{0.1}$ are believed to induce more pronounced toxic effects in humans due to their enhanced capabilities to penetrate deeply into our body system. They also have a greater surface area per unit mass, thus enabling them to adsorb more toxic compounds as compared to their larger counterparts. As mutations may lead to the development of various diseases, including cancer, this study aimed to evaluate the mutagenicity of organic and inorganic air samples of PM_1 and $PM_{0.1}$. **Methods:** Air sampling was conducted by using the Nanosampler Model 3180 in UKM Kuala Lumpur, Malaysia. Following extraction, the mutagenicity of organic and inorganic air samples PM_1 and $PM_{0.1}$ was then evaluated using Ames test without and with metabolic activation on three different strains of bacteria, which were *Salmonella typhimurium* TA98, TA100 and *Escherichia coli* (*E. coli)* WP2. **Results:** There is no mutagenic activity observed in all samples. However, the mutagenicity potential of organic air sample is higher than inorganic air sample on *Salmonella typhimurium* TA98 and TA100 while inorganic air sample showed higher mutagenicity potential in *E. coli* WP2. Based on size, fine particles (PM_1) showed higher mutagenic potential than ultrafine particles (PM0.1). **Conclusion:** Therefore, it can be concluded that organic and inorganic air sample as well as PM_1 air sample have the potential to cause mutations. Future studies focus on the investigation of chemical composition that responsible to the mutagenicity of PM_1 and $PM_{0.1}$ are warranted.

Keywords: Ames test; Inorganic air samples; Organic air samples; $PM_{0.1}$, PM_1

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INTRODUCTION

Air pollution occurs when specific compounds in the atmosphere exceed certain limits, resulting in adverse effects on human health, animals, and plants (1). It consists of various compounds, including carbon monoxide, lead, nitrogen oxides, ground-level ozone, sulfur dioxide, and particulate matter (PM). PM is a complex mixture comprising solid particles and liquid droplets suspended in the air, composed of both organic and inorganic compounds (2). The quality of today's air is deteriorating due to the expansion of industrialization, the increase in private car numbers, and the combustion of fossil fuels. Additionally, air pollution is known to be influenced by weather conditions and the season of a country (3). In Peninsular Malaysia, the climate is divided into four seasons which are Northeast Monsoon, Southwest Monsoon and two interchange monsoons (4). The highest concentration of particulate matter is typically observed during the Southwest Monsoon, also referred to as the hot season in Malaysia (5). The seasonal variation in pollutant production is closely linked to meteorological factors such as rainfall and wind direction (6).

The study of air pollutants or mainly PM gain a lot of attention because it is often linked with human health problems (7). Generally, PM can be divided into distinct fractions based on their aerodynamic diameter. Coarse particles (2.5-10 μm) can infiltrate the human respiratory system and become trapped in the upper respiratory tract. Conversely, particles smaller than 2.5 μm pose greater risks, as they can deeply penetrate the lower respiratory system (8). PM_1 and ultrafine

particles (also known as $PM_{0.1}$), are particles with aerodynamic diameter of ≤ 1 μm and ≤ 0.1 μm, respectively (9). Due to its smaller size ranges, PM_1 and $PM_{0.1}$ are able to bypass the body's natural defences in the upper respiratory tract, enabling them to penetrate deeply into the respiratory system and even enter the bloodstream (9). The majority of detrimental impacts of air pollution on human health are correlated with respiratory ailments, including asthma, acute bronchitis, allergic rhinitis, and upper respiratory tract infections (URTI), since PM are particularly prone to inhalation by living organisms (7).

PM_1 and $PM_{0.1}$ are thought to elicit more pronounced toxic effects on human health due to their reduced aerodynamic size. Furthermore, particles with smaller aerodynamic diameters exhibit a higher surface area per unit mass in comparison to their larger counterparts (9). This increased surface area possesses the ability to adsorb a wide range of hazardous substances such as heavy metals, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs), thereby amplifying their adverse impact when inhaled. Particularly noteworthy are certain chemicals present in PM_1 and $PM_{0.1}$, like PAHs, which have been documented for their capability to interact with genetic material, leading to DNA damage and mutations (10). Additionally, research has uncovered that although immediate noticeable effects might not arise from acute exposure to ultrafine particles, prolonged exposure spanning extended periods can lead to the accumulation of genetic impairments, consequently heightening the susceptibility to mutations (9). Consequently, there is an urgent need to examine the mutagenicity of the PM_1 and $PM_{0.1}$ mutagenic potential of PM_1 and $PM_{0.1}$, given that mutations possess the capacity to disrupt normal cellular processes and potentially give rise to various diseases, including cancer. Moreover, studies focus on the mutagenicity of ultrafine particles remain scarce, especially within tropical regions. Thus, this study aimed to evaluate the mutagenicity of organic and inorganic air samples of PM_1 and $PM_{0.1}$ using Ames test, both with and without metabolic activation.

MATERIALS AND METHODS

Air sampling

The detailed methodology on air sampling has been reported by Jamhari et al. (11). In general, the samples collection took place between 17 February 2017 and 3 December 2017, and pooled samples were employed for assessing cytotoxicity and genotoxicity in this study. The sampling procedure aimed to reflect typical tropical urban air conditions adjacent to a busy main road, with high traffic volume, without considering seasonal fluctuations. Specifically, air samples were collected from the rooftop (approximately 15 m above ground level) of the UKM Kuala Lumpur campus building, situated alongside the road (10 m) on Jalan Raja Muda Abdul Aziz (30 10′ 7.931″ N, 1010 42′ 4.343″ E). This region experienced frequent congestion in traffic flow, especially during mornings and late afternoons, due to its vicinity to major roads—Jalan Tunku Abdul Rahman (250 m) and Jalan Tun Razak (1.2 km).

The Nanosampler's inlet utilized a single-channel design with unidirectional air flow, operating at a flow rate of 40 L/min for a duration of 120 hours (11). The experimental set-up at the sampling sites had the impactor and pump housed in a steel electrical cabinet, with an inlet at the top of the housing covered by a rain hood. The Nanosampler 3180 was installed with PM_{10}, $PM_{2.5}$, PM_1, $PM_{0.5}$ and $PM_{0.1}$ 55 mm quartz fibrous filter (Pallflex 2500QAT-UP) and an inertial filter containing SUS stainless steel fibre wool (9.8 ± 0.03 mg) (Nippon Seisen Co. Ltd., felt type, SUS-304) supplied by the manufacturer (12). This configuration permitted the capture of various-sized PM on their respective quartz fibrous filters, while $PM_{0.1\text{-}0.5}$ were gathered within the inertial filter. To mitigate potential organic residue contamination, all filters were wrapped with aluminium foil and pre-baked at 400 °C for 4 hours in a furnace before sampling. The quartz filter paper and inertial filter were preconditioned in a desiccator at a relative humidity of 35% ± 5% for 48 hours before and after sampling. Subsequently, these filters were weighed using a precision electronic microbalance (Sartorius, USA) with a reading accuracy of 0.001 mg to ensure measurement precision (5). To prevent cross-contamination, each filter was individually wrapped in aluminium foil, sealed within airtight zip-lock PE bags, and stored at 4°C until the extraction of organic and inorganic compounds.

Extraction of organic and inorganic compounds

Prior to the extraction, the filters, retrieved from cold storage, were allowed to equilibrate to room temperature, and any surface moisture was eradicated through evaporation. In brief, the filter paper was cut into pieces and put in the pre-baked glassware. Subsequently, the samples were subjected to ultrasonic agitation with a mixture of dichloromethane (DCM) and n-hexane in a 1:1 v/v ratio, in a series of 15 cycles lasting two minutes each, interspersed with one-minute intervals of rest. After repeating the sonication process twice, the resulting extracts were combined. Before proceeding to the clean-up phase, the extracts were concentrated to approximately 200 μL through gentle nitrogen blowdown. Then, the RP-18 cartridge underwent pre-conditioning with 10 mL of n-hexane prior to loading with the organic sample. The target compounds were entirely eluted from the column utilizing a 10 mL solution of DCM:n-hexane (35:65 v/v) as the eluting agent. The acquired organic fraction

was subsequently subjected to evaporation under mild N2 flow until complete dryness. The resultant dry residue was then reconstituted using dimethyl sulfoxide (DMSO).

For the aqueous extract, the remaining half of each filter paper was cut into pieces and dissolved in 20 mL of distilled water within a 50 mL glass conical flask. This extraction process extended over a period of 60 minutes, facilitated by an ultrasonic bath (Elmasonic S70H, Elma, Germany). Following this, a 10-minute centrifugation at 2500 RCF (Kubota 5100, Japan) was conducted, followed by filtration through an Acrodisc® Supor® membrane filter. Subsequently, the resultant organic and inorganic extracts of PM_1 and $PM_{0.1}$ were stored at -20 °C until the time of analysis.

Growth curve of bacteria

The growth curve of bacteria was constructed to determine the optimal optical density values of bacteria at the appropriate time. To begin, 12 µL of bacteria were inoculated from frozen stock and transferred into a sterilized conical flask containing 12 mL of nutrient broth. This mixture was then incubated at 4°C for 7 hours. Subsequently, the conical flask was moved to a shaking water bath set at a temperature of 37°C for an incubation period lasting 12-14 hours. After this incubation, 250 µL of the bacterial suspension was transferred into 250 mL of nutrient broth. Then, a total of 2.5 mL of the bacterial suspension was pipetted into a cuvette, and the optical density (OD) was measured at 660 nm using a spectrophotometer. This initial reading served as the baseline (0 hours). The bacterial suspension incubated again at 37°C in the shaking water bath. OD readings were subsequently recorded every 2 hours until the 24-hour mark. Upon completing the readings, a graph plotting OD values against time was generated.

Genetic analysis

Genetic analysis was carried out to examine genetic profiling and spontaneous mutation rates in frozen cultures, following the protocol outlined in Test Guideline No. 471 (Bacterial Reverse Mutation Test) as stipulated in the OECD Guidelines for the Testing of Chemicals. In summary, the bacterial cultures were pre-cultured overnight before proceeding with the genetic analysis. Confirmation of genotypes was performed on the *Salmonella typhimurium* TA98 and TA100 mutant strains, encompassing aspects such as histidine/biotin dependence, rfa marker, uvrB gene deletion mutation, and the presence of plasmid pMK101. Similarly, the Escherichia coli (*E. coli*) WP2 strain underwent genotypic testing for tryptophan and uvrA deletion. The outcomes of the genetic analysis are presented in Tables I and II.

Table I : Genetic analysis for *Salmonella typhimurium* TA98 and TA100

Strain	Dependence on histidine	Dependence on biotin	*uvrB* mutation	*rfa* mutation	Presence of plasmid pKM101
Salmonella typhimurium TA98	/	/	/	/	/
Salmonella typhimurium TA98	/	/	/	/	/

Table II : Genetic analysis for *Escherichia coli*

Strain	Dependence on tryptophan	*uvrA* mutation
Escherichia coli	/	/

Table III : Result of Ames test on strain TA98, TA100 and *Escherichia coli*

Air samples	Bacterial strains					
	TA98		TA100		*E. coli*	
	Without S9 (–S9)	With S9 (+S9)	Without S9 (–S9)	With S9 (+S9)	Without S9 (–S9)	With S9 (+S9)
PM_1 (Organic)	–	–	–	–	–	–
PM_1 (Inorganic)	–	–	–	–	–	–
$PM_{0.1}$ (Organic)	–	–	–	–	–	–
$PM_{0.1}$ (Inorganic)	–	–	–	–	–	–

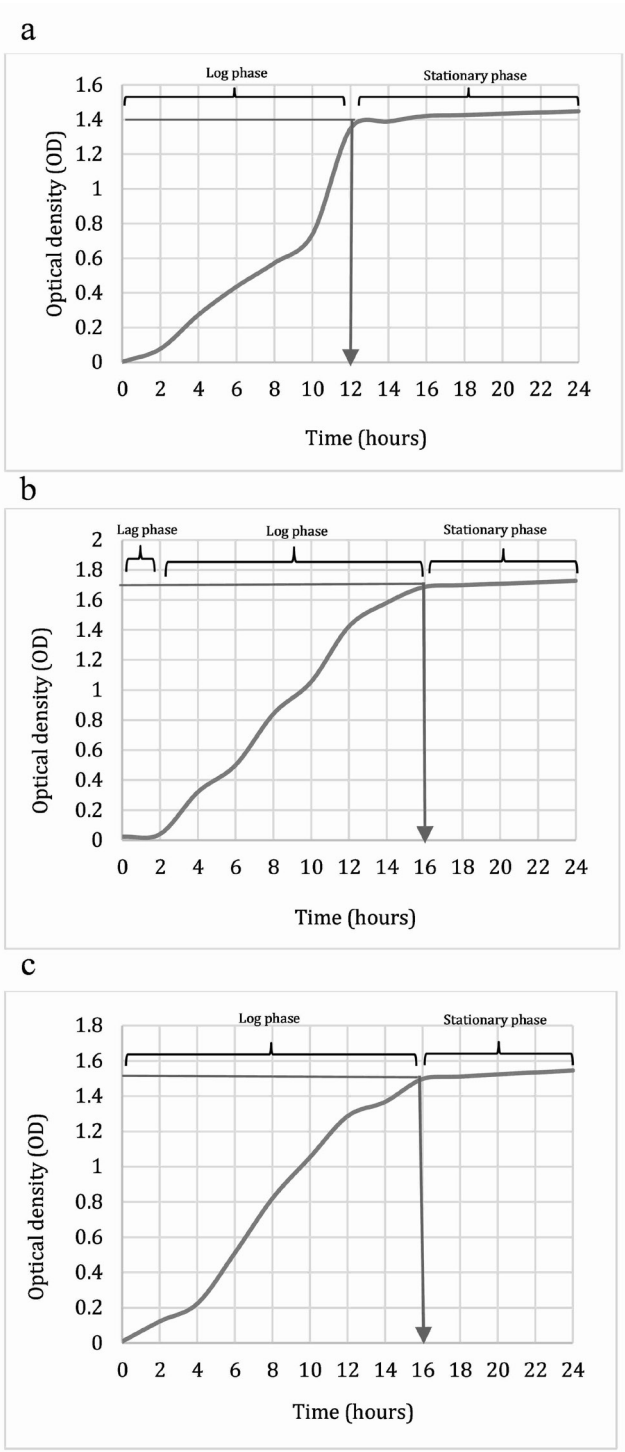

Fig. 1 : The growth curve of (a) TA98 strain, (b) TA100 strain and (c) *E. coli* bacteria used in Ames test.

Mutagenicity assessment

The mutagenicity of the samples was assessed using the Ames test, following the procedure outlined in Test Guideline No. 471 (Bacterial Reverse Mutation Test) as specified in the OECD Guidelines for the Testing of Chemicals. This test employs the pre-incubation method and involves three distinct bacterial strains, which are *Salmonella typhimurium* TA98, TA100 and *E. coli* WP2. Furthermore, the test was conducted both with and without metabolic activation (+S9 and –S9) by incorporating the S9 metabolic activation system into the experimental setup. As bacteria lack metabolic capacity, the inclusion of the S9 mixture (Moltox, Boone, NC, USA) facilitates the detection of potential promutagens, as certain compounds exhibit mutagenic properties only after undergoing metabolism by phase 1 drug-metabolizing enzymes (13).

Briefly, 0.1 mL of the organic and inorganic extract of PM_1 and $PM_{0.1}$ at the concentration of 25%, 50% or 100%, were preincubated with 0.1 mL of test bacterial strain (approximately $1x10^8$ viable cells) and 0.5 mL of sterile buffer (for without metabolic activation setting) or S9 mixtures (for setting with metabolic activation) for 20 minutes at 30-37°C. The tubes were agitated throughout the pre-incubation period by using a shaker. Then, 2 mL of overlay agar was added into the tubes and the mixtures were poured onto the surface of a minimal agar plate. 4-Nitro-o-phenylediamine (2.5 µg/plate), sodium azide (5 µg/plate) and methyl methanesulfonate (2.5 µg/plate) was used as positive control for Ames test without metabolic activation on TA98, TA100 and *E. coli* WP2 strain, respectively. As for the test with metabolic activation, 2-aminoanthracene (0.5 µg/plate) was used as positive control for all the bacterial strains. The negative control of the study was sterile normal saline. The plates were then incubated at 37°C for 48 hours. The determination of mutagenicity was based on the number of revertant colonies formed for each strain following the incubation period. A sample was deemed mutagenic if the count of revertant colonies exceeded double the count of revertant colonies in the negative control. (14,15).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 25.0 (IBM Corp, Armonk, New York, USA), and statistical significance was considered if $p<0.05$. The data were presented as mean ± standard error of mean (SEM), and the Mann-Whitney U test was employed to determine any significant differences between the means.

RESULTS

Fig. 1 shows the growth curve for each bacterial strain. Lag phase is the stage where the bacteria are adapting to the new environment culture that will take a few minutes to a few hours. However, there was no lag phase shown on the bacterial growth curve of TA98 and *E. coli*. This can occur as the bacterial cultures are taken from the cultures that are on the exponential phase and placed in new media under same growth conditions like the temperature and the shaker speed (16). Based on the growth curve, the beginning of stationary phase for both bacterial strain TA98 was

at 12th hours while for strain TA100 and *E. coli* is at 16th hour. This phase is the phase at which the activity of bacterial cells at maximum level and is use for the Ames test.

The genetic analysis of bacteria confirmed that the mutated *Salmonella typhimurium* strains were histidine and biotin dependence, carry rfa and uvrB mutation as well as has plasmid pKM101 (Table I). For the *E. coli,* it was tryptophan dependence and carry uvrA mutation (Table II). Result on Table III show that all the air samples collected around the area of Universiti Kebangsaan Malaysia, Kuala Lumpur did not show any mutagenic activity based on the Ames test. The mutagenicity test on *Salmonella typhimurium* TA98, TA100 and *E. coli* WP2 strain showed no revertant colonies exceed the double number of colonies on negative control. This mean that organic and inorganic air samples of different sizes do not possess mutagenic activity that can cause frameshift or base substitution mutations.

Table IV show that the revertant colony of organic $PM_{0.1}$ air sample were significantly higher ($p<0.05$) as compared to inorganic $PM_{0.1}$ air sample, either with or without metabolic activation. For the sample tested with TA100, there were significant differences ($p<0.05$) in the revertant colony between the organic (167±1.50) and inorganic (151±0.50) PM_1 air samples at 50% concentration, in the present of metabolic activation. For the *E. coli* WP2 strain, at the 50% concentration, the revertant colony of inorganic PM_1 air samples (80±4.50) showed significantly higher ($p<0.05$) mutagenic potential compared to organic PM_1 air samples (40±1.50) without metabolic activation system. Referring to the results of the comparison of mutagenicity potential between organic and inorganic samples for all three bacterial strains, most of the significance difference can be seen at the concentration of 50%. This finding shows that the concentration of 50% is the effective concentration in producing the maximum response. There was no result for $PM_{0.1}$ air samples that to be tested on *E.Coli* strain due to very limited samples.

The comparison of the mutagenic potential of air samples based on size of particulate matters showed that the PM_1, which have larger diameter than $PM_{0.1}$, showed significantly higher ($p<0.05$) mutagenic potential on strains TA98 and TA100 in the absence of metabolic activation. The significant difference ($p<0.05$) in the mutagenic potential of the inorganic samples can be seen at the concentrations of 25% for TA98 strain (21±0.00 vs 12±2.00) and 100% (175±3.00 vs 159±0.50) for TA100 strain. Meanwhile, significant difference ($p<0.05$) in the mutagenic potential of the organic samples can be seen at the concentration of 50% (163±2.50 vs 138±1.50) for TA100 strain.

Table IV : Mutagenicity of organic and inorganic air samples on strain TA98, TA100 and *E. coli*

Metabolic activation	**Without S9 (–S9)**			**With S9 (+S9)**		
Concentration (%)	**25**	**50**	**100**	**25**	**50**	**100**
Strain TA98						
PM_1 (Organic)	20±5.00	20±1.00	23±4.50	27±1.50	29±2.00	23±8.50
PM_1 (Inorganic)	21±0.00#	18±4.00	21±0.00	31±2.00	22±2.50	27±3.00
$PM_{0.1}$ (Organic)	24±1.00*	18±2.50	25±4.50	29±3.50	34±1.50*	14±2.00
$PM_{0.1}$ (Inorganic)	12±2.00*#	19±2.00	21±0.50	23±0.00	23±0.00*	31±10.00
Strain TA100						
PM_1 (Organic)	150±14.00	163±2.50#	147±14.00	152±5.00	167±1.50*	168±8.50
PM_1 (Inorganic)	171±15.50	162±6.50	175±3.00#	174±17.50	151±0.50*	161±9.00
$PM_{0.1}$ (Organic)	125±19.50	138±1.50#	160±1.50	157±7.00	171±7.00	210±21.0
$PM_{0.1}$ (Inorganic)	132±9.00	157±7.50	159±0.50#	144±7.00	162±19.50	169±4.00
Strain *E. coli*						
PM_1 (Organic)	39±9.00	40±1.50*	43±9.00	47±1.50	52±8.00	49±3.50
PM_1 (Inorganic)	68±4.00	80±4.50*	74±6.00	75±2.00	76±4.00	77±7.50
$PM_{0.1}$ (Organic)	-	-	-	-	-	-
$PM_{0.1}$ (Inorganic)	-	-	-	-	-	-

*Significant differences ($p<0.05$) between organic and inorganic samples within the same PM. #Significant differences ($p<0.05$) between PM_1 and $PM_{0.1}$ within the same organic or inorganic samples.

DISCUSSION

The mutagenicity test on *Salmonella typhimurium* TA98, TA100, and *E. coli* WP2 strain showed that no revertant colonies exceeded twice the number of colonies observed in the negative control. Based on our current findings, it can be suggested that both the organic and inorganic extracts of PM_1 and $PM_{0.1}$ do not possess mutagenic activity that could lead to frameshift or base substitution mutations. Numerous previous studies have indicated that organic extracts of PM exhibit mutagenic activity, whether in the presence or absence of metabolic activation (17). Furthermore, Lemos et al. demonstrated that organic extracts have a high mutagenic potential, particularly for fine particles (18). Nonetheless, the disparities between our current findings and previous reports might be attributed to variations in the components present in the air samples collected during different seasons. Past research has illustrated that the level of mutagenicity is elevated during cold or winter seasons (19,20). The higher genotoxicity and mutagenicity of PM collected in these colder months could be attributed to the slower wind speeds and reduced rainfall, which lead to decreased dispersion of PM in the air. Additionally, the increased mutagenicity might be linked to higher concentrations of PAHs during the cold season, as heating becomes an additional source of emissions (10). Hence, the variations in meteorological factors such as wind and rainfall, along with changes in anthropogenic activities due to seasonal shifts, could be responsible for the discrepancies in mutagenic activities observed in the collected PM samples (6).

In Peninsular Malaysia, the climate is categorized into four distinct seasons, which are Northeast Monsoon, Southwest Monsoon and two interchange monsoons (4). Our earlier findings have indicated variations in the levels of inorganic and carbonaceous components, as well as PAHs, across these seasons (5,11). The Northeast Monsoon season registered the highest concentrations of total organic carbon (OC) and elemental carbon (EC), while the Southwest Monsoon season exhibited elevated levels of water-soluble inorganic ions (WSII) and PAHs. However, our current analysis did not account for seasonal fluctuations, as we combined samples collected throughout the entire year for mutagenicity assessment. Therefore, it becomes important to assess the mutagenic potential of PM samples collected during different seasons, considering the variable composition of PM samples across seasons (6). Moreover, as our collected samples are representative of urban air with high traffic volume, we hypothesized that the mutagenicity of these air samples would surpass that of samples collected in rural areas, due to the presence of more hazardous components, such as PAHs released into the air from vehicle fuel combustion (10). Prior studies have shown significantly higher mutagenicity levels in air samples collected at roadways compared to those from suburban areas as well (21).

Our current findings demonstrate that, in the presence of metabolic activation, the number of revertant colonies for organic PM_1 and $PM_{0.1}$ air samples was significantly higher compared to their inorganic counterparts, specifically for the TA98 and TA100 bacterial strains, respectively. The heightened mutagenicity observed in the organic extracts of PM_1 and $PM_{0.1}$ when metabolic activation was involved could be attributed to the chemical components present in the organic fraction of the collected PM samples, particularly substances like PAHs. PAHs belong to a well-known class of chemicals capable of inducing mutations and are often found in urban environments, particularly where combustion processes are prevalent (10). PAHs are classified as procarcinogens and are not inherently mutagenic in their original form, as they do not directly interact with DNA. However, these PAH molecules undergo transformation into electrophilic metabolites through metabolic activation systems like the cytochrome P450 enzymes when entering into our body. In this transformed state, they can exert mutagenic or carcinogenic effects within living organisms (22). Consequently, the increased mutagenicity observed in organic extracts when the S9 metabolic system was present could be attributed to the presence of PAHs in the PM samples. Indeed, our earlier reports indicate the presence of PAHs in both PM_1 and $PM_{0.1}$ air samples (11). Our current findings are further corroborated by a study conducted by Santes et al., wherein results demonstrated that PAHs exhibited significantly higher mutagenic activity, approximately 5 to 7 times higher, when activated by human enzymes (23).

Furthermore, the organic extracts of $PM_{0.1}$ exhibited a notably higher level of mutagenicity when compared to the inorganic extracts, even in the absence of metabolic activation. This observation implies the existence of alternative chemical components capable of directly inducing mutations in the bacterial strain. Alongside PAHs, the organic extracts of PM also encompass other classes of chemical components, such as aldehydes and amines, which could potentially contribute to mutagenic effects (24,25). Therefore, the mutagenicity associated with the organic extracts of PM samples might also stem from the presence of unidentified chemical constituents within the organic fractions.

In the absence of metabolic activation, the number of revertant colonies in the *E. coli* strain treated with inorganic PM samples exceeded those treated with organic air samples at a concentration of 50%. This outcome could be due to the presence of water-soluble elements within PM, including transition metals like iron, copper, and nickel. These metals can act as catalysts for generating reactive oxygen species (ROS) through Fenton-like reactions, thereby increasing the likelihood of inducing oxidative DNA damage in comparison to organic compounds (26). The generated ROS, including hydroxyl radicals, have the potential to directly impair DNA through oxidative modifications and strand breaks (27). If not adequately repaired, these DNA lesions can potentially give rise to mutations. Prior research has demonstrated that water-soluble transition metals present in PM can indeed inflict DNA damage in individuals exposed to such particles, implying a connection between metal content and genetic consequences (28). However, it should be noted that we do not have data on the water-soluble constituents of the PM samples. Hence, future studies are needed to ascertain the specific chemical components, particularly the metal contents within the organic extracts of PM samples, that contribute to the observed mutagenicity in this study.

Our current findings reveal that PM_1 air samples exhibited a greater mutagenic potential on strains TA98 and TA100, in contrast to $PM_{0.1}$. However, our results are in contrary to some previous studies who reported that mutagenic potential increase with decreasing particle size of PM (29,30). The widely accepted notion has been that finer particulate matter tends to possess higher toxicity (9). Particles with smaller aerodynamic diameters usually offer a larger surface area per unit mass in comparison to their larger counterparts, thereby enhancing their capacity to adsorb more harmful substances (9). Moreover, $PM_{0.1}$ has been demonstrated to be capable of translocating transcellularly across lipid bilayers of cell walls via diffusion, potentially intensifying its toxic effects in comparison to larger-sized PM (9). However, besides the particle size, the chemical components should also be taken into consideration while assessing the mutagenicity of the PM samples using the Ames test (31). As per our earlier reports, PM_1 contains higher levels of carbonaceous materials and PAHs compared to $PM_{0.1}$ (5,11). In fact, Topinka et al. demonstrated that ultrafine particles carried fewer PAHs and formed fewer DNA adducts than PM_1 (32). Additionally, the inorganic extract of PM_1 was reported to contain higher concentrations of water-soluble inorganic ions (WSII) than the inorganic extract of $PM_{0.1}$ (5). Therefore, the higher mutagenicity observed in PM_1 in comparison to $PM_{0.1}$ could potentially due to variations in chemical components as well.

CONCLUSION

In summary, the evaluation of mutagenicity concerning the organic and inorganic extracts from PM_1 and $PM_{0.1}$, using *Salmonella typhimurium* TA98, TA100, and *E. coli* WP2 strains, with or without metabolic activation, revealed the absence of revertant colonies surpassing double the number of colonies observed in the negative control. Thus, our current findings indicate the lack of frameshift or base substitution mutations induced by the air samples collected in the UKM Kuala Lumpur campus building. This study also unveiled distinctions between organic and inorganic PM extracts in terms of mutagenic potential. Notably, organic PM extracts exhibited a greater potential to induce mutagenic activity in *Salmonella typhimurium* TA98 and TA100 strains, while inorganic PM extracts exhibited higher mutagenic potential in *E. coli* WP2 strain. Furthermore, the evaluation of mutagenic potential based on the aerodynamic diameter of PM indicated that PM_1 displayed a higher mutagenicity potential than ultrafine particles ($PM_{0.1}$). These findings contribute insights into the mutagenic potential of fine and ultrafine particles gathered in an urban environment characterized by a typical tropical climate.

Future studies should focus on investigating the specific chemical compositions responsible for the mutagenicity of PM_1 and $PM_{0.1}$. Such investigations hold considerable significance in expanding our understanding of the mutagenic properties of fine and ultrafine particles.

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REFERENCES

1. Kampa M, Castanas E. Human health effects of air pollution. Environmental Pollution. 2008;151(2):362–7.
2. Landkocz Y, Ledoux F, Andrй V, Cazier F, Genevray P, Dewaele D, et al. Fine and ultrafine atmospheric particulate matter at a multi-influenced urban site: Physicochemical characterization, mutagenicity and cytotoxicity. Environmental Pollution. 2017;221:130–40.
3. Cichowicz R, Wielgosiński G, Fetter W. Dispersion of atmospheric air pollution in summer and winter season. Environ Monit Assess. 2017;189(12):605.
4. Suhaila J, Mohd Deni S, Zawiah Wan Zin W, Aziz Jemain A. Trends in Peninsular Malaysia Rainfall Data During the Southwest Monsoon and

Northeast Monsoon Seasons. Sains Malaysiana. 2010;39(4):533-42.
5. Jamhari AA, Latif MT, Wahab MIA, Hassan H, Othman M, Abd Hamid HH, et al. Seasonal variation and size distribution of inorganic and carbonaceous components, source identification of size-fractioned urban air particles in Kuala Lumpur, Malaysia. Chemosphere. 2022;287:132309.
6. Shelton S, Liyanage G, Jayasekara S, Pushpawela B, Rathnayake U, Jayasundara A, et al. Seasonal Variability of Air Pollutants and Their Relationships to Meteorological Parameters in an Urban Environment. Levy I, editor. Advances in Meteorology. 2022;2022:5628911.
7. Agarwal AK, Singh AP, Gupta T, Agarwal RA, Sharma N, Rajput P, et al. Mutagenicity and Cytotoxicity of Particulate Matter Emitted from Biodiesel-Fueled Engines. Environ Sci Technol. 2018;52(24):14496–507.
8. Xing YF, Xu YH, Shi MH, Lian YX. The impact of PM2.5 on the human respiratory system. Vol. 8, Journal of Thoracic Disease. Pioneer Bioscience Publishing; 2016. p. E69–74.
9. Schraufnagel DE. The health effects of ultrafine particles. Exp Mol Med. 2020;52(3):311–7.
10. Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum. 2016;25(1):107–23.
11. Jamhari AA, Latif MT, Wahab MIA, Othman M, Hamid HHA, Tekasakul P, et al. Size-segregated atmospheric polycyclic aromatic hydrocarbons down to $PM_{0.1}$ in urban tropical environment: Temporal distribution, potential sources and human health risk. Urban Clim. 2021;40:100996.
12. Furuuchi M, Choosong T, Hata M, Otani Y, Tekasakul P, Takizawa M, et al. Development of a Personal Sampler for Evaluating Exposure to Ultrafine Particles. Aerosol Air Qual Res. 2010;10:30–7.
13. Vijay Urvashi, Gupta S, Mathur P, Suravajhala P, Bhatnagar P. Microbial Mutagenicity Assay: Ames Test. Bio Protoc. 2018;8(6):e2763.
14. Cariello NF, Piegorsch WW. The Ames test: The two-fold rule revisited. Mutation Research/Genetic Toxicology. 1996;369(1):23–31.
15. Ooi TC, Ibrahim FW, Ahmad S, Chan KM, Leong LM, Mohammad N, et al. Antimutagenic, cytoprotective and antioxidant properties of ficus deltoidea aqueous extract in vitro. Molecules. 2021; 26(11):3287.
16. Hall BG, Acar H, Nandipati A, Barlow M. Growth Rates Made Easy. Mol Biol Evol. 2014;31(1):232–8.
17. Viras LG, Athanasiou K, Siskos PA. Determination of mutagenic activity of airborne particulates and of the benzo[α]pyrene concentrations in Athens atmosphere. Atmospheric Environment Part B Urban Atmosphere. 1990;24(2):267–74.
18. Lemos AT, Coronas MV, Rocha JAV, Vargas VMF. Mutagenicity of particulate matter fractions in areas under the impact of urban and industrial activities. Chemosphere. 2012;89(9):1126–34.
19. Bonetta S, Bonetta S, Schilirт T, Ceretti E, Feretti D, Covolo L, et al. Mutagenic and genotoxic effects induced by PM0.5 of different Italian towns in human cells and bacteria: The MAPEC_LIFE study. Environmental Pollution. 2019;245:1124–35.
20. Abou Chakra OR, Joyeux M, Nerriиre E, Strub MP, Zmirou-Navier D. Genotoxicity of organic extracts of urban airborne particulate matter: An assessment within a personal exposure study. Chemosphere. 2007;66(7):1375–81.
21. Kawanaka Y, Matsumoto E, Wang N, Tsuchiya Y, Yun SJ, Jiang ZW, et al. Mutagenic Activity of Atmospheric Ultrafine Particles at a Roadside Site and a Suburban Site. Journal of Health Science. 2006;52(4):352–7.
22. Moorthy B, Chu C, Carlin DJ. Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer. Toxicological Sciences. 2015;145(1):5–15.
23. Santes-Palacios R, Camacho-Carranza R, Espinosa-Aguirre JJ. Bacterial mutagenicity of selected procarcinogens in the presence of recombinant human or rat cytochrome P4501A1. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2018;835:25–31.
24. Thapa MJ, Fabros RM, Alasmar S, Chan K. Analyses of mutational patterns induced by formaldehyde and acetaldehyde reveal similarity to a common mutational signature. G3 (Bethesda). 2022;12(11):jkac238.
25. Nohmi T, Watanabe M. Mutagenicity of carcinogenic heterocyclic amines in *Salmonella typhimurium* YG strains and transgenic rodents including gpt delta. Genes and Environment. 2021;43(1):38.
26. Zhao T, Yan Y, Zhou B, Zhong X, Hu X, Zhang L, et al. Insights into reactive oxygen species formation induced by water-soluble organic compounds and transition metals using spectroscopic method. Journal of Environmental Sciences. 2023;124:835–45.
27. Juan CA, de la Lastra JM, Plou FJ, Pйrez-Lebeca E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. Int J Mol Sci. 2021;22(9): 4642.
28. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metal Toxicity and the Environment. In: Luch A, editor. Molecular, Clinical and Environmental Toxicology: Volume 3: Environmental Toxicology.

Basel: Springer Basel; 2012. p. 133–64.
29. Sicre MA, Marty JC, Saliot A, Aparicio X, Grimalt J, Albaiges J. Aliphatic and Aromatic Hydrocarbons in the Mediterranean Aerosol. Int J Environ Anal Chem. 1987;29(1–2):73–94.
30. Pagano P, De Zaiacomo T, Scarcella E, Bruni S, Calamosca M. Mutagenic Activity of Total and Particle-Sized Fractions of Urban Particulate Matter. Environ Sci Technol. 1996;30(12):3512–6.
31. Park CG, Cho HK, Shin HJ, Park KH, Lim H Bin. Comparison of Mutagenic Activities of Various Ultra-Fine Particles. Toxicol Res. 2018;34(2):163–72.
32. Topinka J, Milcova A, Schmuczerova J, Krouzek J, Hovorka J. Ultrafine particles are not major carriers of carcinogenic PAHs and their genotoxicity in size-segregated aerosols. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2013;754(1):1–6.